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Bacterial Surface Translocation: a Survey and a Classification

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INTRODUCTION

The swarming of strains of *Proteus*, *Bacillus*, and *Clostridium* has been known to bacteriologists for as long as it has been common practice to culture bacteria on solid substrates. Likewise, gliding motility of myxobacteria, blue-green algae, and other microorganisms is a long-recognized phenomenon. In 1961, Lautrop (44) described yet another kind of bacterial motility confined to substrate surfaces, and he later proposed to name it twitching (45). Both swarming, gliding, and twitching lead to the production of what is usually referred to as swarming zones, although for reasons which will be obvious later on they will be called spreading zones in this paper. A spreading zone is a film, broad or narrow, of one or, at most, a few layers of cells extending from the edge of a colony or an area of confluent growth and, therefore, frequently so thin that it is barely visible to the naked eye.

During a study of twitching it became evident that spreading zones may be produced on surfaces of solid media in other ways than the three above-mentioned and so, in order to establish a sound basis for the further study of different kinds of surface translocation and to utilize the different kinds of surface translocation in the characterization of bacteria, it was deemed necessary to try, as far as possible, to detect, describe, define, and classify all possible kinds of surface spreading produced during bacterial growth on solid media. This might also help to clarify what value such characters have in taxonomy.

Six different types of surface translocation have been recognized so far: (i) swarming, dependent on excessive development of flagella and partly on cell to cell interaction; (ii) swimming, dependent on flagella and fluid; (iii) gliding, dependent on intrinsic motive forces and partly on cell to cell interaction; (iv) twitching, dependent on intrinsic motive forces (and fimbriae?); (v) sliding, dependent on growth and reduced friction (i.e., spreading by expansion; and (vi) darting, dependent on

growth in capsulated aggregates (i.e., spreading by ejection).

The macroscopic appearance of a spreading zone will only sometimes be so characteristic that it directly reveals how the zone was produced. This may, however, in all cases be decisively established by examining the spreading zone under the microscope.

In the following text, a survey is presented of the different kinds of bacterial surface translocation based on the micromorphological features of the spreading zone pattern and the manner of single-cell movement. The author's observations are reported in each section after a review of the pertinent literature, and each section ends with a discussion and the author's definition of the kind of surface spreading in question.

MATERIALS AND METHODS

Bacterial Strains

Table 1 lists the species and the number of strains of each species examined so far during the author's study. Strains specifically mentioned or depicted, or both, in this paper are listed in column 2 in Table 1.

Inspection of the Surface Colonies

Because spreading zones may be inconspicuous, the use of a hand lens is essential.

Agar Plate Microscopy

A Carl Zeiss photomicroscope with phase contrast equipment and a 6-mm auxiliary condenser lens was used. Most of the observations were made with low-power, dry lenses (×16, ×25, or ×40), but occasionally an oil immersion objective was also used. In such cases the observations were also checked with a dry lens, because placing a cover slip on top of the agar plate culture inevitably alters the conditions by introducing another surface (glass) and extra fluid. Photomicrographs were taken with a photomicroscope by using an electronic flash (Ukatron UN 60) and Kodak Tri-X films.

Wet Mounts

Wet mounts were used to reveal flagellar motility in appropriate fluid media.

Determination of Velocity Values

Single cells, aggregates of cells, or the leading edge of the spreading zone were followed directly under the microscope. Distance covered was measured by means of an eyepiece graticule and time was recorded with a stop watch.

Staining of Flagella

The technique of Leifson (47) was used.

Media

Meat extract agar plates (MEA) were made of 0.5% meat extract, 1.0% peptone (Orthana), 0.3% sodium chloride, 0.2% primary sodium, and 1.8% agar.

Ascites agar plates (AA) were made of two volumes of MEA (with an agar concentration of 2.2%) and one volume of ascitic fluid.

The 5% blood-agar plates were made of MEA mixed with 5% (v/v) defibrinated horse blood.

The 10% blood-agar plates were made of MEA mixed with 10% (v/v) defibrinated horse blood.

Cytophaga agar plates were used in the following modifications. No. 62 was made of 0.05% tryptone (Difco) 0.05% yeast extract (Difco), and 1.0% agar (Difco), with a final *p*H adjusted to 7.0. No. 70 was made as no. 62, but with tryptone concentration of 0.5%. "Ordal" was made of 0.2% tryptone (Difco), 0.05% beef extract (Difco), 0.05% yeast extract (Difco), 0.02% sodium acetate, and 1.0% agar (Difco), with a final *p*H adjusted to 7.2 (55).

Incubation

Clostridium tetani was incubated in an anaerobic jar (Baird and Tatlock, Ltd.). With the exception of *Proteus mirabilis* and *Bacillus alvei* the rest of the strains were incubated aerobically in plastic bags in order to ensure a humid atmosphere. The temperatures used were either 22, 30, 33, or 35 C, as indicated in the text or the legends.

Nomenclature

In this paper the term "surface translocation" is synonymous with the terms "surface spreading" and "motility on a solid surface." The term "swarming" as usually employed in the literature covers any surface spreading phenomenon but is here always qualified as "swarming in the broad sense" when used in this way. "Swarming in the narrow sense" or just "swarming" is here only used about swarming as specifically defined later in this paper.

OBSERVATIONS AND DISCUSSION

Swarming

Many excellent and detailed observations of swarming (i.e., swarming in the narrow sense) of bacteria can be found in the literature, and a complete review of the many papers will not be given; only certain facts pertinent to the problem of flagella-dependent surface motility in contrast to other spreading phenomena will be discussed. Classical swarming is shown by *P.*

TABLE 1. *List of strains examined*

Species studied	No. of strains studied	Designation of selected strains[a]	Origin of selected strains[b]
Acinetobacter calcoaceticus	265	BD-4	Juni
Acinetobacter calcoaceticus		17905	ATCC
Acinetobacter calcoaceticus		AB 156	SSI
Alcaligenes odorans	6	H 1079	SSI
Bacillus alvei	1	HV 56	SSI
Bacillus anthracis	10	Ax 11	Thal
Bacillus cereus	3	HV 57	SSI
Bacillus circulans	1	HV 61	SSI
Chondrococcus columnaris	1	1-R 43	Ordal
Clostridium tetani	2	An 551/71	SSI
Clostridium novyi	1	An 73/70	SSI
Corynebacterium sp.	1	HV 59	SSI
Cytophaga succinicans	1	RL-8	Ordal
Cytophaga sp.	5	U 67	SSI
Escherichia coli	11	W 3703 Hfr	Ørskov
Flavobacterium meningosepticum	12	13253	ATCC
Flavobacterium sp.	30	U 120	SSI
Kurthia zopfii	3	8603	NCIB
Moraxella bovis	3		
Moraxella kingii	3		
Moraxella nonliquefaciens	7		
Moraxella osloensis	34	A 249	SSI
Moraxella phenylpyruvica	1	23633/69	Frederiksen
Moraxella urethralis[c]	1		
Proteus mirabilis	10	H 1093	SSI
Proteus vulgaris	10		
Pseudomonas acidovorans	2		
Pseudomonas aeruginosa	13	AB 1421	SSI
Pseudomonas alcaligenes	10	PR 389	SSI
Pseudomonas mallei	15	23344	ATCC
Pseudomonas maltophilia	2		
Pseudomonas pseudoalcaligenes	69		
Pseudomonas stutzeri	2		
Pseudomonas sp.	1		
Salmonella newport	1		
Salmonella typhimurium	1		
Simonsiella crassa	1	UWO 367	Murray
Staphylococcus albus	3	HV 54	SSI
Streptococcus pneumoniae	9		
Streptococcus sp. (nonhemolytic)	15	4932/71	Frederiksen
Vitreoscilla sp.	2	UWO 390	Murray

[a] Indicates the designation of the strain that is specifically mentioned or depicted in this paper.

[b] ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; Frederiksen, W. Frederiksen, Department of Medical Bacteriology, County Hospital of Aalborg, Aalborg, Denmark; Juni, E. Juni, Department of Microbiology, University of Michigan, Ann Arbor, Michigan, U.S.A.; Murray, R. G. E. Murray, Department of Bacteriology and Immunology, University of Western Ontario, London, Canada; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; Ordal, E. J. Ordal, Department of Microbiology, School of Medicine, University of Washington, Seattle, Wash., U.S.A.; SSI, Department of Diagnostic Bacteriology, Statens Seruminstitut, Copenhagen, Denmark; Thal, E. Thal, Statens Veterinärmedicinska Anstalt, Stockholm 50, Sweden; Ørskov, Ida and F. Ørskov, International Escherichia Centre (WHO), Statens Seruminstitut, Copenhagen, Denmark.

[c] An unofficial name, see reference 45a.

mirabilis and *P. vulgaris* (33, 38, 53), by some species of *Bacillus* (see references 54 and 69 for reviews of the literature), and by some species of *Clostridium*, notably *C. tetani* and *C. novyi* (22, 82).

All swarming organisms possess peritrichous flagella and are able to swim in fluid media. Clark (13) states that nonflagellated variants of *B. alvei* have lost the ability to swarm. Surface-active substances in certain concentrations inhibit swarming and swimming in liquid media of *Proteus* by impairing the production

of flagella (18, 42), and, furthermore, inhibition of the swarming of *P. mirabilis* by ethylenediaminetetraacetic acid (EDTA) has recently been reported to be associated with inhibition of the synthesis of flagella and prevention of the formation of the long-cell forms (87). The one serotype of *C. tetani* that does not swarm is nonflagellated (74). Ørskov (54a) describes how the activity of the flagella of a proteus swarmer cell moving on an agar surface may be convincingly demonstrated by means of his "direct India ink-agar microscopy," because the active flagella fling the ink particles away from the cell.

Most flagellated bacteria, however, will not swarm (in the narrow sense) and even motile, i.e., swimming, but nonswarming strains of *Proteus* have been described (15). These strains could be made to swarm by treatment with phage lysates of other phenotypically similar strains (14), indicating that something more than the presence of normally functioning flagella, i.e., flagella capable of producing swimming motility, is needed for an organism to swarm. All available evidence demonstrates that the special requirement is an excessive number of flagella. Thus, Roberts (64) describes a swarming *B. rotans* as having numerous flagella; Hoeniger (39) shows photographs of long cells of *P. mirabilis* having more than 1,000 flagella per cell; Turner and Eales (82) describe the long, filamentous cell forms of the swarming groups in the clostridia as being "richly endowed with luxuriant flagella"; and also, Boltjes (9) provides evidence that swarming bacteria have a large number of flagella. The curious developmental cycle of swarming strains of *Proteus* (38, 53) is a characteristic and unique feature of these organisms, but the *long* swarmer cells have their counterpart in the *filamentous*, heavily flagellated cells that constitute the advancing edge of swarming strains of *Clostridium* (82).

It is another characteristic of swarming that the cells move in the form of large rafts (53) or "bullet-shaped" colonies (54), whereas isolated cells almost never move. The size of the rafts or motile microcolonies is clearly dependent on the amount of available moisture (73). The drier the agar, the bigger the rafts or the microcolonies. On insufficiently dried agar, individual cells may be seen to move, whereas rotating and wandering colonies of *Bacillus* are best demonstrated on either very dry plates or on plates with a high concentration of agar. Lowering the agar concentration results in diffuse spreading (37). Willis and Williams (89) have shown that inhibiting the swarming of *C. tetani* by adding antitoxin to the plates results in the appearance of motile daughter colonies at the periphery of the nonswarming colonies. Small, wandering colonies arising from the main colony were seen with *Proteus* grown on nutrient agar containing homologous O serum (67, 68).

Murray and Elder (54) conclude that "swarming is an expression of motility under special conditions—in the thin fluid layer overlying the gel." "The swarming of *Proteus* is a consequence of the movement of long cells and this movement is only continuous when one cell is in contact with another," state Morrison and Scott (53). And these authors advance the hypothesis "that the flagella of these very long, heavily flagellate, cells are able to obtain a thrust on neighbouring cells which gives them sufficient impetus to propel themselves over the surface of a solid medium."

As examples of different kinds of swarming, observations on *P. mirabilis*, *C. tetani*, and *B. alvei* are described.

Figure 1 shows the appearance of an agar plate 24 hr after streaking with a strain of *P. mirabilis* (to the far left in the picture) and incubating at 30 C. The typical, terraced spreading reflecting the cyclic growth and swarming of such organisms is clearly seen. Under the same conditions some strains will swarm extensively, covering the entire plate within 24 hr, whereas other strains only produce narrow spreading zones. The reason for this variation is unknown. The spreading rate of a swarming strain depends upon the humidity of the plate, upon the growth rate of the organism, and thus upon the incubation temperature.

The outermost part of the spreading zone seen in the microscope during active swarming is depicted in Fig. 2. The cells, roughly measuring from 10 to 30 μm, are mainly arranged in large bundles, or rafts, with areas of cell-free agar surface in between. Under ordinary laboratory conditions (i.e., the agar surface not being too moist) motility is confined to the rafts and isolated cells lie still. The pattern, as seen in the microscope, is constantly changing, the rafts moving quickly along in continuous curves at a rate of approximately 10 to 15 μm/sec. The isolated cells in a raft may move in opposite directions, and this enhances the impression of high speed. For the sake of convenience, the velocity values determined by me are compiled in Table 2 where also all the values are converted into μm/min for comparative purposes.

Flagella staining of the swarmer cells reveals an enormous number of flagella per cell (inset to Fig. 2). In broth, the long swarmer cells, as

well as the shorter nonswarming cells, swim at the same speed of 10 to 15 μm/sec. When the plates are insufficiently dried, isolated swarmer cells will be seen to move, although not as fast as rafts of cells, and the rate of spreading may be so accelerated that the whole plate is covered in one wave. On the other hand, wandering colonies may be seen under conditions that tend to inhibit or slow down swarming as, for instance, on rather dry agar plates incubated at 22 C. Presumably, this is because the motile power of groups of cells is bigger than that of single cells and probably, to a certain extent, depends on the number of cells in the group.

The very delicate film of swarming produced by a strain of *C. tetani* is seen in Fig. 3, which is a photograph of a 10% blood-agar plate taken after 24 hr of incubation at 35 C in an anaerobic jar (Baird and Tatlock, Ltd.). Because the spreading does not occur in cycles, it may be very difficult to observe the film and determine its expanse. Using reflected light and scraping off the bacteria with a platinum loop will help observation. As with all other kinds of spreading, the rate of swarming of *Clostridium* depends on the dryness of the agar and the growth rate of the organisms. On ordinary laboratory media the swarming of *C. tetani* is so regular and characteristic that it can be used for the isolation of such strains from heavily contaminated material. This was realized as early as 1925 by Fildes (22).

Figure 4 shows the appearance of the spreading film as seen in the microscope. The very long cells that measure up to 50 μm are arranged in narrow bundles. Due to the extreme sensitivity to oxygen of these organisms I have only been able to observe the last trace of the subsiding motility of the cells, which takes

TABLE 2. *Compilation of approximate velocity values for the different spreading phenomena*[a]

Type of spreading	Name of organism[b] and designation of strains in parentheses	Rate of movement of cell rafts or aggregates in μm/min	Rate of progression of edge of spreading zone in μm/min[c]
Swarming[d]	*P. mirabilis* (H 1093)	700	75
	C. tetani (An 551/71)	—	50
	B. alvei (HV 56)	60	20
Swimming[d]	*B. cereus* (HV 57)	—	50
Gliding	*Vitreoscilla* sp. (UWO 390)	15	7
	C. columnaris (1-R 43)	5	3.5
	Cytophaga sp. (U 67)	70	15
Twitching	*A. calcoaceticus* (ATCC 17905)	5	5
	P. alcaligenes (PR 389)	4	4
Sliding	*A. odorans* (H 1079)	—	5
	Flavobacterium sp. (U 120)	—	10
	A. calcoaceticus (BD-4)	—	10
	Streptococcus sp. (4932/71)	—	3
	B. anthracis (Ax 11)	—	2
Darting	*Staphylococcus albus* (HV 54)	—	6

[a] The values vary with the strains and the conditions, which are those described in the text.

[b] The nonabbreviated generic names may be found in Table 1.

[c] These values should be compared with the value of 0.33 μm/min representing the rate of increase in colony radius of *Escherichia coli* on a defined medium at 37 C (59).

[d] The swarming and swimming strains swim in wet mounts at velocities between 600 and 1,000 μm/min.

FIG. 1. *Proteus mirabilis strain H 1093 culture plate incubated on meat extract agar at 30 C for 18 hr;* ¾ *natural size.*

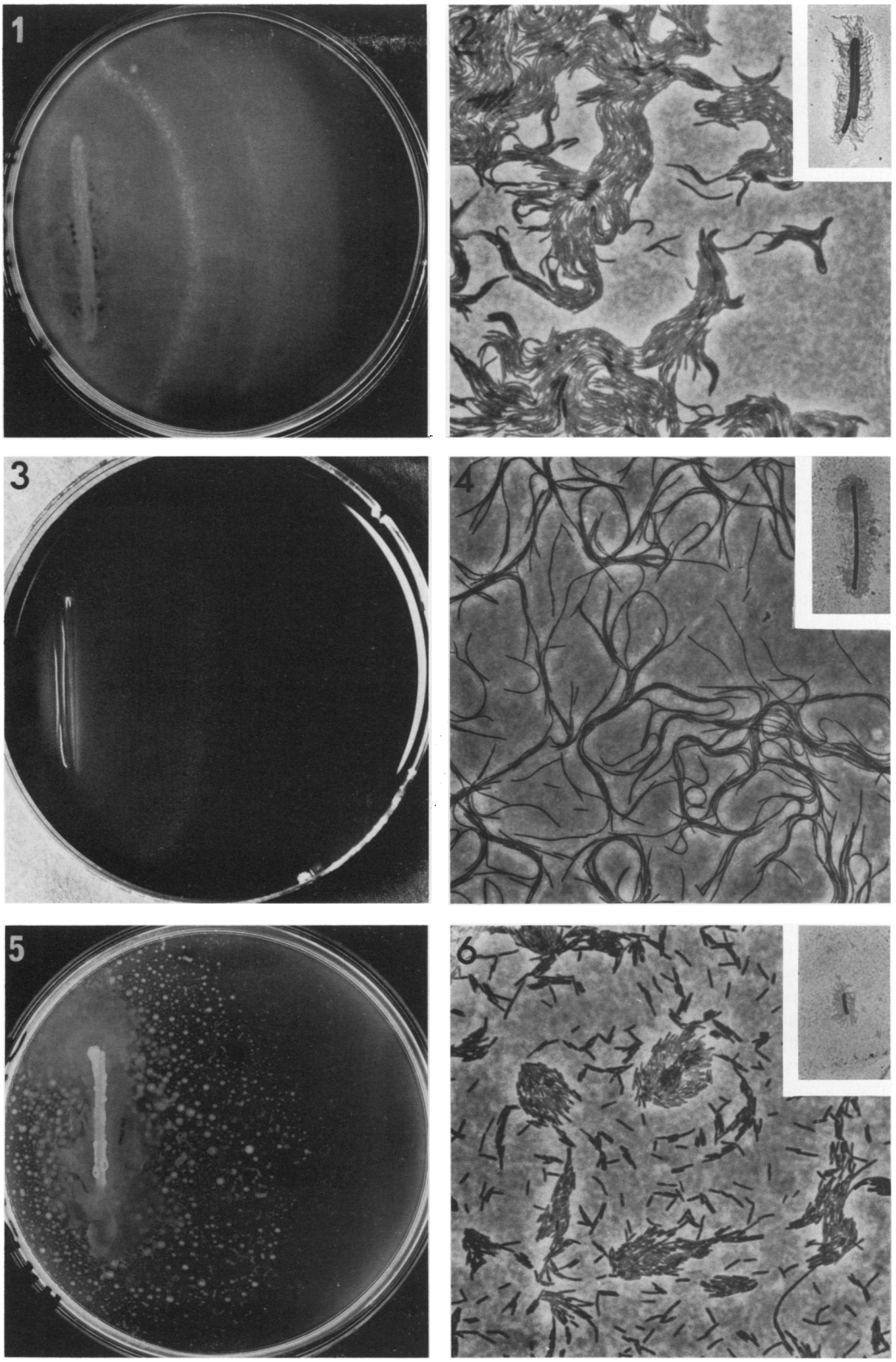
FIG. 2. *Swarming. Peripheral part of swarming zone in Fig. 1, ca. ×300. Inset: swarm cell doubled up and with numerous flagella. Flagella stain; ×600.*

FIG. 3. *Clostridium tetani strain An 551/71 culture plate incubated on 10% blood agar at 35 C for 18 hr;* ¾ *natural size.*

FIG. 4. *Swarming. Same strain as in Fig. 3 but incubated on ascites agar at 35 C for 18 hr. Shows peripheral part of swarming zone, ca. ×200. Inset: long swarm cell with a fur of flagella. Flagella stain; ×600.*

FIG. 5. *Bacillus alvei strain HV 56 culture plate incubated on meet extract agar at 22 C for 48 hr;* ¾ *natural size.*

FIG. 6. *Swarming. Peripheral part of swarming zone in Fig. 5, ca. ×300. Inset: flagella stain of heavily flagellated cell from swarming zone; ×600.*



FIGS. 1–6.

place in the cell bundles. A flagella stained cell is shown in the inset to Fig. 4. Because of the large number of flagella the individual flagellum does not present itself clearly, but rather the cell looks as if being wrapped in a fur of flagella.

The very fascinating swarming of certain species of *Bacillus* that also exhibit wandering and rotating colonies is examplified in Fig. 5. This figure shows the swarming of a *B. alvei* strain on MEA after 48 hr of incubation at room temperature. Single colonies are easily seen in the swarm, and in the microscope many such colonies are seen to rotate. The tracks of the curved paths taken by the, usually smaller, wandering colonies are rather inconspicuous on a photograph of this size. This strain swarms on the ordinary laboratory plate media, but the rate of spreading and the appearance of the swarm depend very much upon the humidity of the plate. The rotating and wandering colonies are only seen clearly with the naked eye when the surface of the agar is relatively dry (or the agar content of the medium very high). Using only lightly dried agar plates and incubating such plates in a humid atmosphere the swarming will be faster and appear as a thin film.

Fig. 6 shows the microscopic picture of the spreading zone where "bullet-shaped" microcolonies, rafts, and single cells are lying in a loose pattern, leaving large areas of agar surface uncovered. By direct observation, motility is seen to be confined to cells in contact with other cells as, e.g., in the rafts which move steadily over the agar surface at a constant speed of about 1 μm/sec. The wandering microcolonies may be much bigger than the ones shown here. The drier the agar, the bigger the microcolonies. They travel in continuous curves and nearly always counterclockwise (seen in the microscope). If a drop of water is put on top of such a colony, it disintegrates immediately, showing that the cells are not bound very tightly together. Eventually a migrating colony might catch up with its own tail and thereby produce a rotating colony that usually does not translocate but only rotates on the spot. A rotating colony is not shown for, at this enlargement, it would have filled the entire field. It should be noted that the cells are short in comparison with the long swarmer cells of *Proteus* and *Clostridium* but that, relatively, they are just as heavily flagellated as these (inset to Fig. 6).

With a slight modification of the "direct India ink-agar microscopy" of Ørskov (54a) further information on the swarming process can be obtained. By placing a drop of diluted India ink on the cell film and a cover glass on top of the drop, so that only one corner covers the drop, variations are often created in the thickness of the layer of fluid in which the ink particles are uniformly distributed, and areas may usually be found where the layer of fluid is sufficiently thick to allow the cells to swim freely. They do so at a speed of about 10 μm/sec and appear in the microscope surrounded by a light halo, produced on the dark background where the particles are being whirled away by the flagella. If, then, a cell swims into an area where the layer of fluid is thinner, the movement changes its character: the cell ceases to rotate and moves steadily along on the agar surface with a somewhat slower speed, and the India ink particles come closer to the body of the cell. The changes observed clearly indicate that the flagella function differently in relation to the surroundings during swarming and swimming.

It should be stressed that the species described are only used as examples of types of swarming which also occur in related species as, *P. vulgaris*, *C. novyi*, and *B. circulans*. The features that these types of swarming have in common are essentially two, viz., the very heavy flagellation of the cells and the fact that the translocation predominantly takes place where cells are in contact with other cells, i.e., in cell bundles, rafts, microcolonies etc. This suggests that the motile power is bigger in cell aggregates than in single cells and this also fits in with the fact that these aggregates become bigger when conditions for swarming become poorer (drying of the agar, for example). In fact, migrating microcolonies can be found under conditions that are suboptimal for swarming in all three types of organisms, although to a varying extent, of course. It seems most likely that there is a gradual transition from the surface motility of a bundle of two to three cells to a macroscopically visible migrating colony.

Smith (74), in writing that "the phenomenon of motile colonies (of *C. novyi*) is not connected with swarming," probably misinterprets Turner and Eales (82) when they say that "the phenomenon (of motile daughter colonies) may and often does occur under conditions unsuitable for swarming." Turner and Eales' statement is, of course, perfectly true but it does not allow for the conclusion that Smith draws.

The difference between the swarming of *Proteus*, *Clostridium*, and *B. alvei* is primarily that the former two have long swarmer cells, while the latter has swarmer cells of "normal" size. One could speculate on whether this has

any relation to the much more pronounced tendency of the *Bacillus* strains to form migrating colonies under conditions suboptimal for swarming because the motile power of the long swarmer cells of *Proteus* and *Clostridium* must be bigger than that of the much smaller *Bacillus* cells.

The delimitation of swarming from swimming is based on the observation that swarming motility is qualitatively different from swimming motility and should be looked upon as a special kind of bacterial locomotion dependent on the presence of a surface and with the propulsive force generated by the many flagella functioning in an as yet unknown manner.

The delimitation of swarming from spreading phenomena other than swimming presents no problems for these phenomena are all independent of flagella and, furthermore, have their own characteristic micromorphological cell pattern and manner of movement.

Definition of swarming. Swarming is a kind of surface translocation produced through the action of flagella but is different from swimming. The micromorphological pattern is highly organized in whirls and bands. The movement is continuous and regularly follows the long axis of the cells which are predominantly aggregated in bundles during the movement.

Swimming

In his studies on chemotaxis in bacteria Adler (1), among other methods, uses semisolid agar plates to examine the influence of different chemicals on the flagellar motility of *Escherichia coli*. With a concentration of 0.2% agar the bacteria swim on top of the agar but may also, if oxidative metabolic pathways are not preferred, swim throughout the depth of the agar or at the bottom. The bacteria are said to swarm, the meaning obviously being swarming in the broad sense (see Nomenclature, p. 479). Gard (24) uses the word "swarming" in the same way when he states that all bacteria which swim in fluid medium will swarm under suitable conditions, i.e., if the concentration of agar is sufficiently low in the plate medium. Such a semisolid agar plate is often called "swarm agar"; a more appropriate designation would be "motility agar." Because the term "swarming" has thus been used about the surface swimming taking place in "swarm agar," it is understandable that confusion has sometimes arisen (see, e.g., references 5 and 48).

The spreading zone of a strain of *B. cereus* produced by swimming motility on the surface of the solid cytophaga agar "Ordal" is shown in Fig. 7. The most peripheral part of the zone is quite delicate and it may—macroscopically—be discerned that it consists of closely set finger-like projections. This spreading is very sensitive to dryness of the medium and for this reason it will only be seen on freshly poured, slightly moist plates or in semisolid motility agar media. Most bacteriologists will know this kind of annoying phenomenon when insufficiently dried media are used and *B. cereus* occurs as a contaminant. Strains which spread like this, however, will also spread on cytophaga agar plates if incubated in plastic bags.

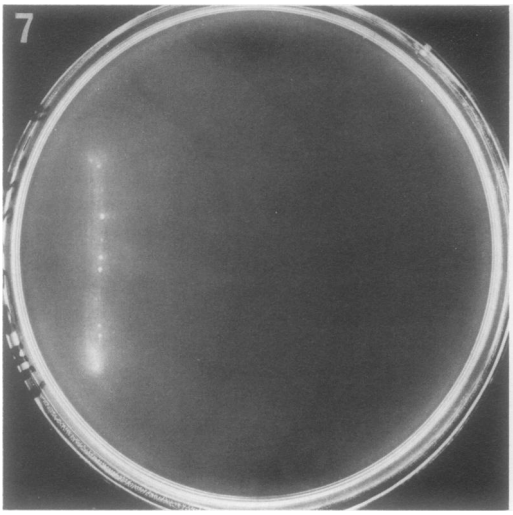
Fig. 8 shows the microscopic appearance of the peripheral part of the finger-like projections. This picture clearly is different from those of *P. mirabilis*, *C. tetani*, and *B. alvei* and is, indeed, produced in an entirely different manner: the individual cells swim freely in the surface film of liquid, little by little pressing projections further out over the agar surface. The inset shows that the cells have peritrichous flagella but not nearly as many as seen on *B. alvei*, for example. Evidently then the only feature which the spreading of *B. cereus* has in common with the swarming of *P. mirabilis*, *C. tetani*, and *B. alvei* is the production of a spreading zone. The differences are that *B. cereus* (i) actually does not move on the agar surface but swims *in* a preformed film of surface fluid of sufficient thickness, (ii) has a relatively small number of flagella, and (iii) does not spread on all media, and this last point is related to the first.

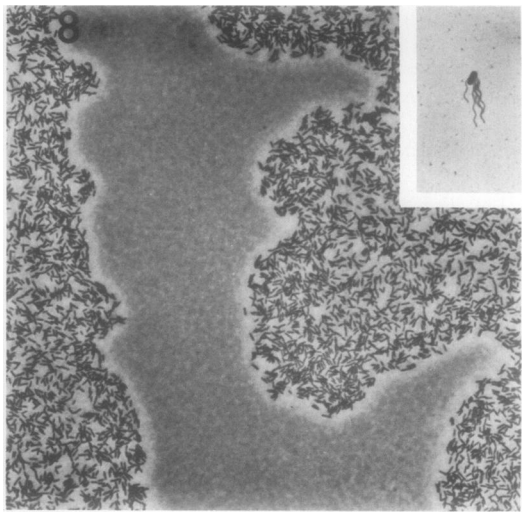
Here it might be appropriate to mention that the spreading so often seen in strains of *Pseudomonas aeruginosa* is not usually, as has been generally assumed, due to surface swimming but is due to twitching. This is apparent in all cases of spreading *P. aeruginosa* examined in this study.

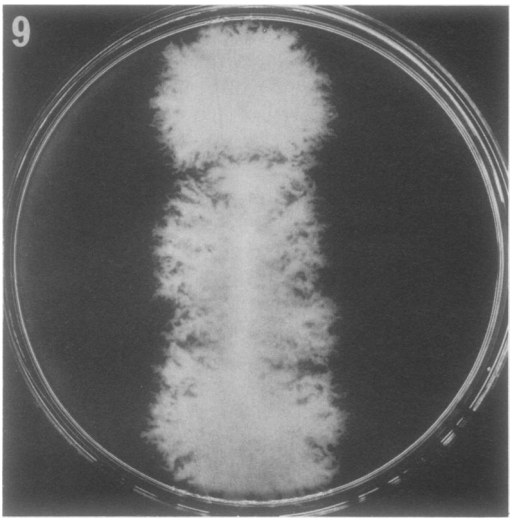
Definition of swimming. Swimming is a kind of surface translocation produced through the action of flagella, but is different from swarming and only takes place when the film of surface fluid is sufficiently thick. The micromorphological pattern is unorganized. The cells move individually and at random in the same manner as flagellated bacteria in wet mounts.

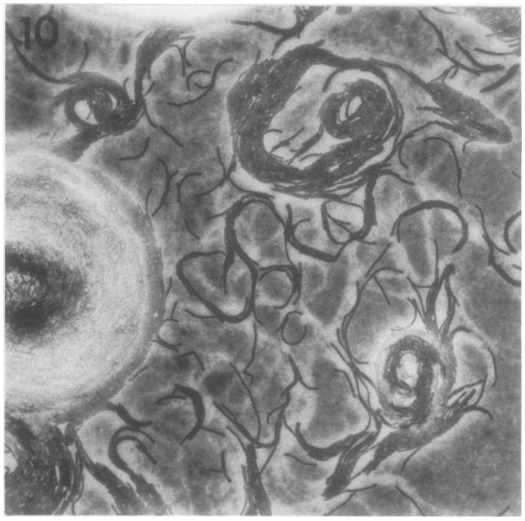
Gliding

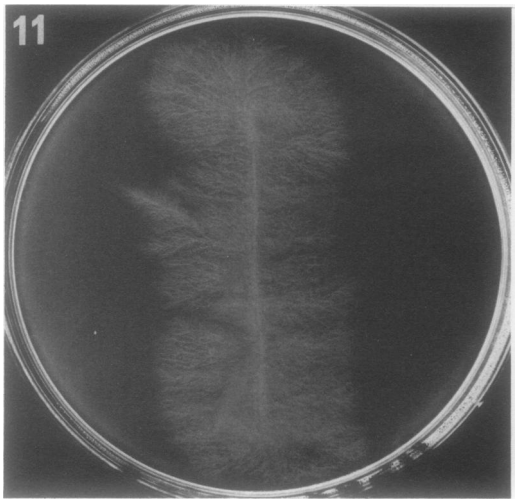
The kind of surface translocation designated gliding has been observed in several groups of microorganisms including some bacteria. It is, however, first of all a characteristic associated

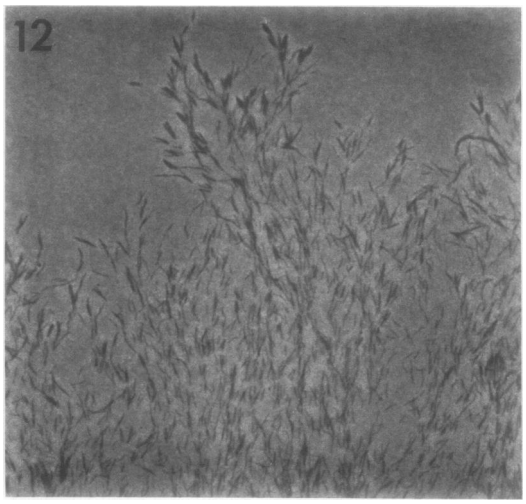












FIGS. 7–12.

with filamentous blue-green algae, but unicellular blue-green algae also may have this property (80).

Among the bacteria, three main groups exhibit gliding motility. According to Stanier et al. (79) they are: (i) filamentous, gliding bacteria consisting of the following principal genera, *Beggiatoa*, *Leucothrix*, *Simonsiella*, *Saprospira*, *Thiothrix*, and *Vitreoscilla*; (ii) fruiting myxobacteria comprising genera such as *Archangium*, *Chondromyces*, *Chondrococcus*, *Myxococcus*, *Podangium*, *Polyangium*, *Sorangium*, and *Stigmatella*; and (iii) the cytophaga group with the genera *Cytophaga* and *Sporocytophaga*.

Besides in these groups of procaryotic cells, gliding has been demonstrated in *Mycoplasma pneumoniae* on glass surfaces in liquid media (4, 10).

Members of the first group of gliding bacteria are now generally accepted as apochlorotic blue-green algae. The literature on the gliding phenomenon in this group is very extensive and will not be surveyed here. Readers are referred to earlier reviews (40, 41, 60, 61, 62, 75).

The descriptions of gliding motility in the two other groups of bacteria are based mainly on observations of suspensions of bacteria in fluid media (ordinary wet mounts or hanging drop preparations) or agar plate cultures, although in individual cases it is not always quite clear on what method the description is based. Most observers agree that gliding motility is confined to solid surfaces, e.g., glass and agar gel but, according to Bisset (8) and Drews and Nultsch (19), the surface need not be solid but might also be the surface film of a fluid (Bisset) or the air-water interface (Drews and Nultsch). In general, observations of wet mounts register the behavior of single cells as opposed to the observation of aggregates of cells in agar plate colonies.

The movements of single cells in wet mounts are described as lashing around a fixed pole, flexing and gliding (49), and "a rolling and darting jerky movement" (16) occurring only when cells are in contact with slide or cover slip. The flexibility of the myxobacterial cell was stressed by Stanier (78) but questioned by Anderson and Ordal (3). Referring to the bending of proteus swarmer cells, Dworkin (20) proposes that flexibility "merely is a consequence of a high length-to-thickness ratio of the cell."

The demonstration of gliding motility on agar surfaces is very dependent on the humidity and much more so than that of swarming. Leaving the petri dish open, so that the film of surface liquid evaporates, leads to the cessation of motility (3). Therefore, the colony morphology of gliding organisms is very much influenced by the amount of moisture present (3, 62), and the concentration of nutrients also plays a decisive role, peptone concentrations higher than 0.25 to 0.5% being inhibitory to the gliding of cytophagas (34, 78). Under conditions optimal for gliding, the colonies will be seen as "completely flat, rapidly spreading, almost invisible swarms" (78) or as a spreading, rhizoid growth with a honeycomb appearance (2). Movement takes place mainly in "spearheads" (i.e., spearhead-shaped cell aggregates at the edge of the colony) (78), single, isolated cells very rarely being motile (76), and the picture is one of a "changing dispersed border" with "interlacing bands being continuously rearranged" (45). On nutrient agar, with the usual concentration of nutrients, the colonies are convex, smooth, and glistening with an entire edge and no sign of spreading (78). Many of the gliding bacteria form rotating microcolonies (21, 29, 62) exhibiting a movement similar to that of rotating microcolonies of certain species of *Bacillus*, except that the direction of rotation is a matter of chance (30) as opposed to the predominantly counterclockwise direction observed in the rotating species of *Bacillus* (54).

The rate of translocation in gliding locomotion varies with the organisms and conditions (see reference 17 for a full review), but it has to be pointed out that, when conditions are not

←FIG. 7. *Bacillus cereus strain HV 57 culture plate incubated on cytophaga agar "Ordal" at 22 C for 18 hr;* ¾ *natural size.*

FIG. 8. *Swimming. Peripheral part of spreading zone in Fig. 7; ca.* ×*200. Inset: flagella stain of cell from spreading zone;* ×*600.*

FIG. 9. *Vitreoscilla sp. strain UWO 390 culture plate incubated on meat extract agar at 30 C for 48 hr;* ¾ *natural size.*

FIG. 10. *Gliding. Peripheral part of spreading zone in Fig. 9. The colony to the left in the picture was slowly rotating when the photograph was taken; ca.* ×*200.*

FIG. 11. *Chondrococcus columnaris strain 1-R 43 culture plate incubated on cytophaga agar no. 62 at 22 C for 96 hr;* ¾ *natural size.*

FIG. 12. *Gliding. Peripheral part of spreading zone in Fig. 11; ca.* ×*200.*

specified, comparisons of velocity values do not make sense. When Stanier (77) is cited for having observed a maximum speed of 150 μm/min for soil cytophagas (17), it should be stressed that the movement in question is not that of the swarming edge outwards, nor is it that of a spearhead or a single cell on an agar surface, but it is the movement seen in wet mounts, under uncontrolled temperature, of single cells over an unspecified distance and for an unknown time. Jarosch (41) gives the value of 6 to 8 μm/sec as the gliding rate of *Beggiatoa* sp. on glass slides at room temperature. The gliding motility of a 10-cell trichome of *Vitreoscilla* on agar, at a rate of 23 μm/min, was followed for 15 min by Costerton et al. (16). By measuring the distance covered by a *Simonsiella crassa* filament on agar at room temperature (after 6 hr of incubation at 37 C) from the pictures of Steed (81), a rate of translocation of 0.42 μm/min can be calculated.

Many theories about possible mechanisms of movement have been put forward during the years (see references 17, 40, 41 and 86 for detailed reviews), but none of the theories have as yet been proven. The most promising approach at present seems to me to be electron microscopy. Some results have already been obtained and because these have not been surveyed before, these results are surveyed below.

Electron microscopy of thin sections of *Vitreoscilla* (16) and of *Myxococcus xanthus* (84) revealed no microscopical structures likely to explain the gliding of these organisms. In 1967 Pate and Ordal (56), however, reported the finding of fibrils intimately associated with the inner layer of the outer-unit membrane of *Chondrococcus columnaris*. The fibrils were seen to run parallel to each other in wide bands, with a center-to-center distance of approximately 16 nm, and they were found both in preparations of lysates and in sections of cells (56). The possible role of such fibrils in gliding motility cannot be assessed until more organisms have been studied by using the same technique of fixation. Schmidt-Lorenz and Kühlwein (72) in 1968 announced the finding of bundles of either filaments 4 to 5 nm thick or tubular structures with diameters of 10 to 16 nm running parallel to the longitudinal axis of the cell. These filaments or microtubuli lie freely in the cytoplasm, i.e., under the plasma membrane and, therefore, cannot be the same as the peripheral fibrils found in *C. columnaris*. They were found in 12 different strains of myxobacteria representing seven different named species and five unnamed species. They had not been found in cells of *Chondromyces* and *Sorangium*. These authors categorically state that these elements are contractile and represent the long-sought motility organelles of myxobacteria. They give no arguments in favor of this hypothesis, however. In a later paper these structures, with no further evidence in favor of their function as locomotor organelles, are called intracytoplasmic flagella (71). On the other hand, comparative fine-structure studies of sections of a strain of *M. xanthus* and a nongliding mutant of this strain showed no differences, suggesting a structural basis for a mechanism of gliding (11).

Cytochalasin B (12) appears to inhibit reversibly the function of contractile microfilaments of many eucaryotic cell types (88), but this drug had no inhibitory effect on gliding motility of three strains of *Cytophaga*. Yet, this might have been due to lack of penetration of the antibiotic into the cells (35).

Additionally, it should be mentioned that a parallel array of fibrils, 5 to 8 nm wide, has been found wrapped around the trichome of the blue-green alga, *Oscillatoria princeps*, and it is suggested that the fibrils actually propel the trichome (31).

Jarosch (41) defines gliding as "the active movement of an organism in contact with a solid substratum where there is neither a visible organ responsible for the movement nor a distinct change in the shape of the organism." Lautrop (45) writes: "Gliding or creeping motility in bacteria is defined as the ability of the organism to perform active translocation when it is in contact with a solid surface. It is implied that the cells have no flagella or any other recognizable organs of locomotion." Both of these definitions are unnecessarily broad, as will be shown below.

To represent the three major groups of gliding bacteria mentioned previously, I have chosen for study: (i) a *Vitreoscilla* species, (ii) *C. columnaris*, and (iii) an unidentified species of *Cytophaga*.

Only gliding motility on agar surfaces will be commented on, for the interpretation of the movements seen in wet mounts is especially complicated due to the influence of a number of factors that are difficult to control, such as the oxygen supply, the thickness of the fluid between the two glass surfaces, the cleanness of these surfaces and, thereby, their physicochemical characteristics.

Figure 9 shows the spreading growth of a strain of a *Vitreoscilla* sp. on MEA after 48 hr of incubation at 30 C in a plastic bag. The spreading zone looks rather massive compared

with that of the other gliding organisms, and this is due to the rich growth on this medium, which would prevent spreading of the two other gliding strains examined here. A photomicrograph taken from the spreading zone is shown in Fig. 10. Trichomes, many of which measure 100 μm or more, are seen to be arranged in rafts and bundles of varying size. Motility tracks appear light on the darker background of the agar. To the left in the Fig. 10 part of a microcolony measuring 200 μm in diameter is seen. This colony rotates slowly. Gliding motility otherwise is mainly seen in the rafts of cells which move at a speed of 15 μm/min on this medium. This should be compared with the rate of approximately 7 μm/min at which the edge of the spreading zone moves peripherally. Gliding continues until the whole plate is covered, in 4 to 5 days' time, from the center of the dish (which measures 9 cm in diameter). The gliding of *Vitreoscilla* is less sensitive to the composition of the medium and the humidity than that of the two other organisms used. On other media, such as the cytophaga agar "Ordal," the microscopy pattern is slightly different, appearing somewhat more organized, and also single trichomes can be seen to move, often for minutes in the same direction, but the speed of the gliding is essentially the same as on MEA. Cells of gliding *Vitreoscilla* strains are nonmotile in wet mounts unless touching the glass surface.

The one strain of *S. crassa* examined by me showed gliding motility of single trichomes (on AA at room temperature after incubation at 35 C for 18 hr) at a speed of only 0.6 μm/min which, however, compares well with the value of 0.42 μm/min calculated from the figure of Steed (81).

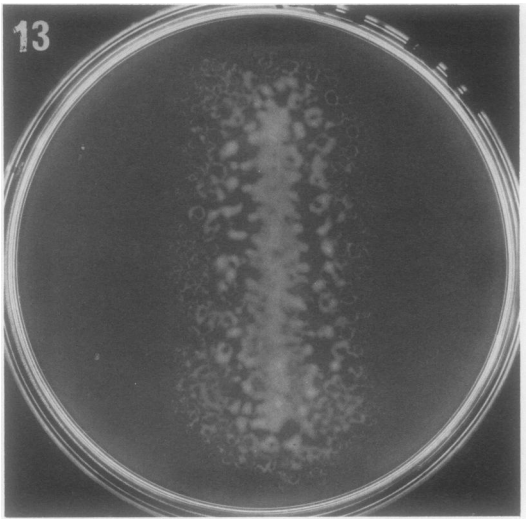
The beautifully structured, feather-like spreading produced by a strain of *C. columnaris* on cytophaga agar no. 62 by means of gliding motility is shown in Fig. 11. This photomicrograph was taken after 96 hr of incubation at 22 C. Under such conditions it takes a week or more to cover the entire agar surface. Spreading does not take place on media with a higher content of nutrients. But on the different modifications of cytophaga agar plates, gliding is a constant trait, although the microscopical appearance of the spreading zones varies considerably. Slime production is not evident on these media. In contrast to other gliding organisms, the growth of this strain adheres to the agar and is difficult to scrape off. The very orderly pattern of cells on medium no. 62 is shown in Fig. 12. The formation of rafts is not predominant, but clearly the cells are arranged in strands and small bundles of five to ten organisms, leaving parts of the agar surface uncovered. Movement is a rather slow but steady gliding in the direction of the long axis of the cells at rates of approximately 5 μm/min under optimal conditions, including incubation in plastic bags to insure a high humidity.

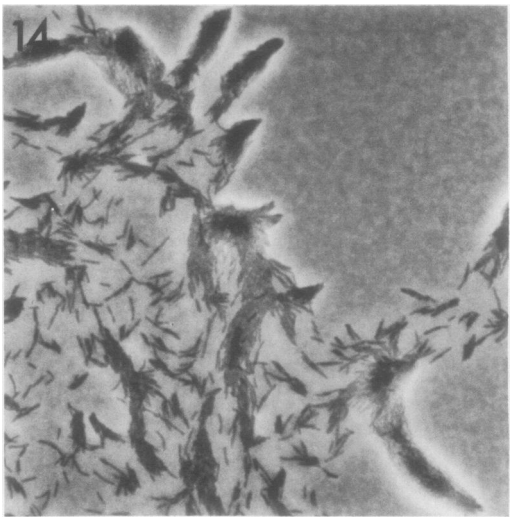
Fig. 13 shows the appearance of a *Cytophaga* sp. gliding on cytophaga agar no. 62. The spreading zone is quite broad, and this strain will, in fact, be able to cover the whole agar surface of the dish in between 2 and 3 days. Figure 14 shows the outermost part of the advancing edge. The cells are arranged in a loose pattern of interlacing bands and rafts of cells, leaving areas of the agar surface uncovered but for single scattered cells. Groups of cells resembling spearheads are seen projecting outwards. The locomotion, which is principally seen in the groups of cells, i.e., rafts and spearheads, and takes the direction of the longitudinal axis of the bacteria, gives rise to a constantly changing picture, steadily gliding groups of cells uniting or dividing and other groups slowly arising from individual cells. Single cells may also be seen to move, the direction again being that of their long axis, but intermittently and more slowly than groups of cells and often reversing or changing direction. Single-cell gliding is seen only if the humidity is kept high. Spearheads of cells from this strain will, under optimal conditions, move along at speeds of 70 μm/min. In comparison, the edge of the spreading zone moves peripherally at a speed of 15 μm/min.

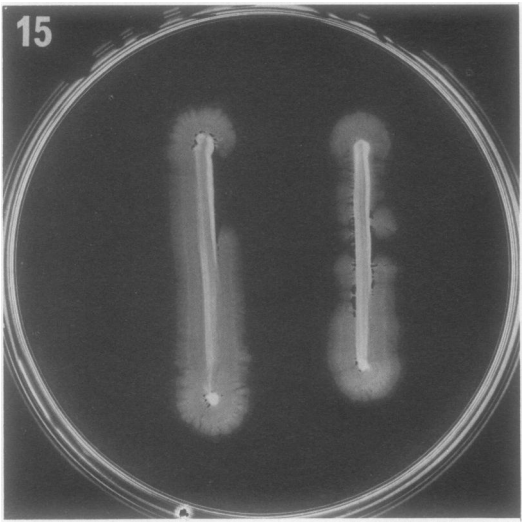
On cytophaga agar no. 62 other *Cytophaga* strains glide, at room temperature, with other velocities and produce spreading zones which might vary slightly in micromorphology but which have in common all the principal features of gliding locomotion just described. Velocity values of 6 and 15 μm/min for the translocation of spearheads of another strain of *Cytophaga* sp. and a strain of *Cytophaga succinicans*, respectively, compared with that of 70 μm/min for the depicted strain, U 67, could possibly be a consequence of a much less pronounced tendency of forming rafts or spearheads in the two slower strains.

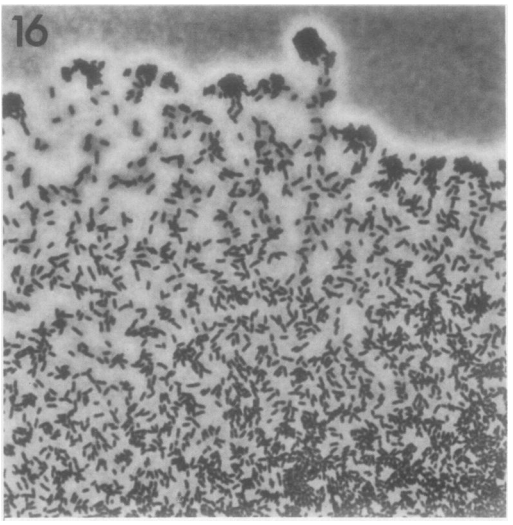
The general microscopical appearance of gliding may, in some cases, be very similar to that of swarming if the difference in speed is disregarded.

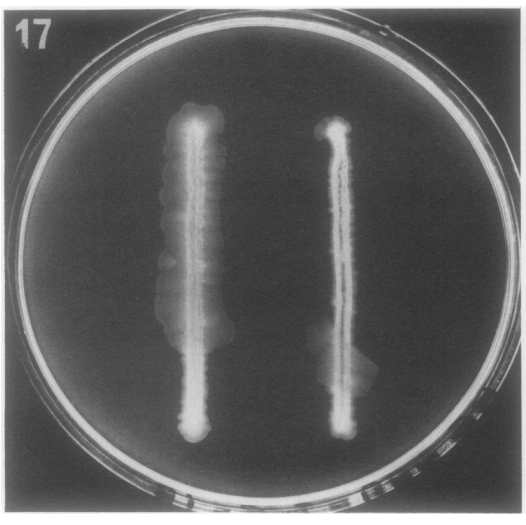
The primary and obvious difference between the two processes, swarming and gliding, of course, is that swarming is performed by bacteria having flagella and gliding is performed by nonflagellated bacteria.

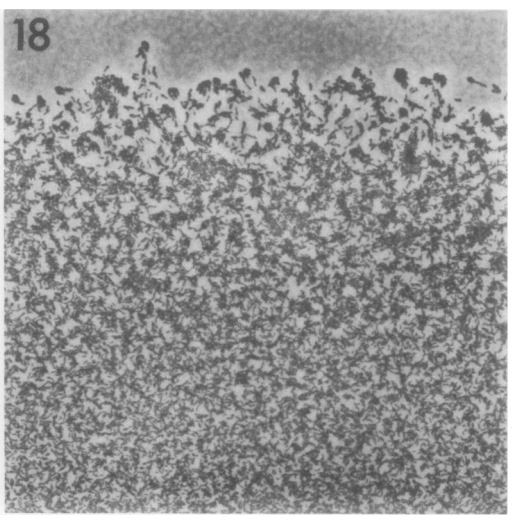












FIGS. 13–18.

But there are other differences. Gliding is much more dependent on the amount of moisture available on the surface of the medium. The trichome-forming bacteria like *Vitreoscilla* will glide on MEA, but the translocation is more pronounced on media which are poorer in nutrients and incubated in a humid atmosphere. And the cytophagas will not glide at all on MEA or similar media in which the peptone concentration is too high, but instead they form distinct colonies with a compact border. With all three cytophaga agar media I found that optimal conditions for gliding also include the use of freshly poured plates and incubation in a humid atmosphere. Neither the concentration of nutrients nor that of agar should be too high. An agar concentration just sufficiently high to prevent the cells from moving down into the agar is optimal.

The delimitation of gliding from sliding and darting is simple, as will be shown later.

Gliding, in contrast to twitching motility, usually results in a spreading growth that eventually covers the entire agar surface. But this is not always so. McMeekin et al. (50) mention at least one strain, allocated to the cytophagas, as exhibiting gliding motility (as judged by direct microscopy of microcolonies) but not spreading growth. Under carefully controlled conditions similar strains, in my experience, will produce at least perceptible spreading zones. But, in any case, it seems quite evident that at present the size of the spreading zone, or rather its continuous propagation, cannot be used to define gliding motility. The differences between gliding and twitching pertain mainly to the characteristic manner of movement of the cells, necessitating a description of this as part of the respective definitions. Returning now to the definitions of gliding given by Jarosch and Lautrop, it can be stated immediately that they cover equally well gliding and twitching. In both cases it is a matter of translocation of nonflagellated bacteria in contact—and only then—with a solid surface. Both definitions use the term "active" in connection with the words "movement" and "translocation," respectively. This might or might not be appropriate; nobody can know with certainty as long as explanations of the two phenomena have not been found. Therefore, I shall omit this term. Also, I prefer to omit any statement about organs of locomotion other than flagella at this state of knowledge. Accordingly, the essential parts of these two definitions, with an additional description of cell movement, will be adequate.

Definition of gliding. Gliding is a kind of surface translocation produced by an unknown mechanism occurring only in nonflagellated bacteria. The micromorphological pattern is highly organized in whirls and bands. The movement is continuous and regularly follows the long axis of the cells which are predominantly aggregated in bundles during the movement.

Twitching

This kind of bacterial, surface-bound motility, independent of flagella, was first demonstrated in strains of *A. calcoaceticus* in 1961 by Lautrop (44) who also, in 1965, named it "twitching" (45). His original findings were soon confirmed by others (26, 29, 32, 57, 70). The same kind of motility is reported to have been found in strains of *M. lacunata* and *M. nonliquefaciens* (57), in *M. bovis*, *M. kingii*, and *M. nonliquefaciens* (36), in nonflagellated strains of *P. aeruginosa* (45), in an unnamed *Moraxella* sp. (6), and in some gram-negative yellow-pigmented rods (50).

Most authors study twitching motility by means of microscopy of cultures on thin agar plates poor in nutrients (i.e., some cytophaga agar modification or other) (29, 32, 44, 50, 70). Remarks about spreading zones are scarce (45, 50), but Gianelli and Cabassi (26) present color photographs of strains of *Acinetobacter* and *Moraxella* with spreading zones varying in width, appearance, and distribution along the central streak of growth. They describe the zones as *Proteus*- and *Bacillus*-like or barely perceptible and looking like acanthus leaves or petals. Unfortunately, these authors do not seem to have examined their plate cultures under the microscope and thus are not in a position to know how the spreading actually took place. Others have used a specially designed "oil chamber" where the bacteria, in a drop of broth, are placed between an oil and an

←FIG. 13. *Cytophaga sp. strain U 67 culture plate incubated on cytophaga agar no. 62 at 22 C for 48 hr;* ¾ *natural size.*

FIG. 14. *Gliding. Peripheral part of spreading zone in Fig. 13; ca.* ×*300.*

FIG. 15. *Acinetobacter calcoaceticus strain ATCC 17905 culture plate incubated on cytophaga agar no. 62 at 30 C for 24 hr;* ¾ *natural size.*

FIG. 16. *Twitching. Peripheral part of spreading zone in Fig. 15; ca.* ×*300.*

FIG. 17. *Pseudomonas alcaligenes strain PR 389 culture plate incubated on cytophaga agar no. 62 at 30 C for 48 hr;* ¾ *natural size.*

FIG. 18. *Twitching. Peripheral part of spreading zone in Fig. 17; ca.* ×*200.*

agar surface (6, 57). The descriptions of single cell movements seen in an oil chamber or on an agar plate surface are consistent with each other. The motility appears as small, intermittent jerks covering only short distances and often changing the direction of movement, which is not regularly related to the long axis of the cell; the maximum speed of a group of cells is about 1 to 2 μm/min (45). The only other velocity value found in the literature is one of 2 to 5 μm/min (29).

Only very little information is available about optimal experimental conditions. Piéchaud (57) considers an agar concentration of 0.5 to 0.7% superior to higher concentrations, and Gianelli and Cabassi (26) recommend the same concentration with Difco agar. The latter furthermore found that higher concentrations of nutrients (1% peptone and 0.5% meat extract) with some strains gave better results than the medium used by Halvorsen (32), which they originally tried. Varying the *p*H from the original 7.2, altering the surface tension by addition of Tween 80, and using other energy sources did not lead to better spreading of their strains (26).

Some misunderstandings need to be corrected. Samuels et al. (66) write: "... we believe that the alleged motility of *Herellea* and *Mima* (now: *Acinetobacter calcoaceticus*) has not been proved. We believe that it is diagnostically correct to refer to *Herellea* and *Mima* as nonmotile organisms, as determined by the usual methods of motility examination in semisolid agar or in hanging drop suspensions." Obviously the misunderstanding arises from the use of the word "motility." The authors do not realize that the motility they refer to in the first of the sentences quoted is twitching motility which cannot be observed by the same methods as swimming motility of flagellated organisms, to which they refer in the last sentence quoted. Therefore the first sentence is false and the last sentence is true.

The mechanism of twitching motility is unknown. There has been a tendency to relate it to another, also unexplained, phenomenon, namely gliding motility (17, 32, 44, 70). Lautrop (45), however, in 1965 presented arguments in favor of treating twitching and gliding as separate taxonomic characteristics. Ryter and Piéchaud (65) concluded from an electron microscopy study, by using both negative staining and thin sections, that the cell wall of *Moraxella* has a structure like that of *Vitreoscilla*, and they proposed that this cell wall structure might be related to the characteristic motility of these organisms. But Lautrop et al. (46), on the other hand, found "a striking overall similarity" in the ultrastructure of 27 species of gram-negative bacteria including, among others, *A. calcoaceticus*, *M. nonliquefaciens*, *P. aeruginosa*, *E. coli* and *P. mirabilis*.

Piéchaud (58) in 1969 suggested that filaments, called "proflagella," were the cause of the twitching motility of *Moraxella*. These filaments apparently are the same as those described by Ryter and Piéchaud earlier (65) as "filaments probablement d'origine capsulaire." No explanation for the mode of action of these structures is proposed but for the allusion to flagellar activity (58). Henrichsen et al. (36) reported that twitching motility and fimbriation are correlated in colony variants of *M. nonliquefaciens*, *M. bovis*, and *M. kingii*, and they conclude that fimbriation most likely is a necessary but not a sufficient condition for twitching motility in these organisms. Twitching motility, like gliding, is not influenced by cytochalasin B (35).

The occurrence of twitching motility seems to be limited to relatively few groups of gram-negative bacteria. Apart from *A. calcoaceticus*, *P. aeruginosa*, and the species of *Moraxella* already referred to above, I have at present only found this kind of surface translocation in strains of *M. osloensis* and in different species of nonfluorescent *Pseudomonas* (J. Henrichsen, *unpublished observations*).

The spreading produced by a twitching strain of *A. calcoaceticus* is shown on Fig. 15. Spreading may occur along the entire streak or, as is often the case, as a varying number of fanshaped zones that may be unevenly distributed. This is not a reflection of a directly and genetically determined inhomogeneity in the bacterial population, since a strain like the one shown here cannot be split up into a spreading and a nonspreading variant. Similarly, single colonies may be diffusely spreading or produce fanshaped offshoots. The rate at which the leading edge moves peripherally is usually 2 to 5 μm/min, but it varies with the strain and the conditions. In some isolates the spreading zone is rather inconspicuous.

Figure 16 shows the typical appearance of the outermost part of the spreading zone as seen in a microscope. In contrast to what is seen with gliding and swarming organisms, the general impression is one of a much less organized distribution of the cells over the agar surface; interlacing strands of cells do not occur, and raftlike cell aggregates are not formed except at the verge of the spreading zone. These cell aggregates, however, only move peripherally with the speed of the advancing edge, and they

are formed at the verge where the movement of the individual cell is arrested, partly because of the collision among cells and partly because the surface film of fluid outside the spreading zone presumably becomes so thin that conditions for twitching motility deteriorate. Within the area of randomly scattered cells, with uncovered agar surface in between, single cells and occasionally small clusters of cells can be seen to move in jerks sideways or in the direction of their longitudinal axis, covering in one jerk distances of up to 2 to 3 μm, but usually much shorter distances. The direction of movement of the individual cells, as well as the number of jerks per time unit, is quite haphazard. Sometimes a small number of cells lying together may be seen to make a jump, in any direction, in a body. With strains that show a high twitching activity it can be seen with a magnification of ×1,000 or more that the cells, also in between the jerks, are continuously moving extremely slowly in shifting directions. From this it will be understood that it does not make sense to try to measure the speed of single cell movements. Just as the spreading zones may be quite narrow, the directly observable twitching activity may be inconspicuous depending on the strain in question and the conditions used for studying the phenomenon.

Some strains of *A. calcoaceticus* produce relatively large amounts of extracellular slime, and the cells may then be seen to lie apparently within a "common capsule" and exhibit what might be called abortive twitching motility not leading to any spreading. In some such cases quite active twitching may be seen if a cover glass is applied.

At least one strain (AB 156) in my collection of twitching *A. calcoaceticus* isolates produces motile microcolonies when twitching motility is partly inhibited by the addition of 10^{-2} M $CaCl_2$ to the medium. Such microcolonies move at a speed of 2 μm/min leaving tracks of scattered single cells behind. The path is curved, but no direction, clockwise or counterclockwise, seems to predominate.

Spreading produced by a twitching strain of *P. alcaligenes* is shown in Fig. 17, and Fig. 18 gives the micromorphology of the spreading zone (note that the enlargements of Fig. 16 and 18 are not the same; they are ×300 and 200, respectively). In comparison with Fig. 15 and 16 the overall impression is one of great similarity. Likewise, the character of the cell movement is covered by the description already given for *A. calcoaceticus*, and the rate of outward locomotion of the verge of the spreading zone is of the same order of magnitude, namely 4 μm/min. In short, this strain also exhibits typical twitching motility. The cells have, however, one polarly inserted flagellum, and normal flagellar activity, i.e., swimming, immediately becomes evident if a drop of fluid medium is placed on the agar surface. Nevertheless, it can be stated with a very high degree of confidence that this spreading is the result of twitching and not of swimming because: (i) nonflagellated variants of strains of different species of *Pseudomonas* produce the same degree of spreading and exhibit the same characteristic single cell movements as flagellated strains, if they spread at all; and (ii) occasionally swimming is seen at the same time as twitching motility, but then it is always located to that part of the spreading zone closest to the densely packed single cell layer (bottom part of photograph in Fig. 18) where surface fluid presumably is most plentiful, whereas twitching alone can be seen peripherally to this. On direct observation the two kinds of cell movement are easy to distinguish.

P. mallei is the only species of *Pseudomonas* that is constitutionally nonflagellated. None of 15 examined strains of *P. mallei* showed twitching motility (J. Henrichsen, *unpublished observations*).

A systematic study of the composition of the plate media showed that twitching, like gliding motility, is very dependent on the availability of moisture, for the greatest activity was observed on media poor in nutrients, with a relatively low agar concentration, freshly poured, dried for a very short period, and incubated in a humid atmosphere (J. Henrichsen, *unpublished observations*). For the same reason it is advantageous to use thick, rather than thin, agar plates, and this does not prevent plate microscopy if only an auxiliary condenser lens is used. This means that for most purposes a modified cytophaga agar, like medium no. 62, will be satisfactory. Most strains of *A. calcoaceticus* exhibiting maximum twitching activity on this medium do not spread at all on MEA.

Some twitching strains, e.g., most species of *Moraxella*, will not grow on medium no. 62, and other media, e.g., AA, must then be tried.

I have not succeeded in isolating spreading and nonspreading variants of twitching *A. calcoaceticus* strains, but twitching and nontwitching wild-type strains of *A. calcoaceticus* have been compared in electron microscopy studies. The comparison shows that twitching strains have fimbriae and that nontwitching strains do not (J. Henrichsen and J. Blom,

manuscript in preparation). It therefore seems to be a necessary condition for twitching motility that the bacteria have fimbriae and presumably a special type of fimbriae. At least no twitching motility could be detected in 11 strains of *E. coli* representing bacteria with at least two different types of fimbriae (three strains were Hfr, one strain was F^+, two strains were hemagglutinating, and the remaining five were nonmotile strains) (J. Henrichsen, *unpublished observations*).

The similarities between twitching and gliding lie primarily in the facts that both types of surface translocation are independent of flagella, are, by and large, dependent on the same cultural conditions, and also are similar in one important feature of the micromorphology of the spreading zone: the cells are scattered over the agar surface. But the patterns are different. That of gliding organisms is highly organized, whereas that of twitching organisms is only slightly so.

Other differences between twitching and gliding motility are found in the speed and total, or final, progression of the spreading and in the characteristic manner of single cell movement. The spreading of a gliding strain usually continues until the entire agar surface is covered by bacteria; the spreading due to twitching motility stops before that. This difference presumably has the following explanation: the speed of progression of the spreading zone in gliding organisms primarily *depends* on the speed of movement of the cell rafts, whereas in twitching organisms the speed of progression of the spreading zone, primarily dependent on growth activity, secondarily *determines* the speed of the movement of the marginal cell aggregates.

The difference between spreading by twitching and by sliding is evident from the microscopic appearance of the spreading zones including the behavior of single cells, but the rates of spreading and the resulting colony, or streak, morphology are closely similar. And it is in fact possible that pronounced sliding may in some cases prevent the demonstration of an existing potential for twitching motility.

Definition of twitching. Twitching is a kind of surface translocation produced by an unknown mechanism, occurring in both flagellated and nonflagellated bacteria, but never due to the action of flagella. The micromorphological pattern is varying but not as organized as in swarming and gliding. The cells move predominantly singly, although smaller moving aggregates occur. The movement appears as intermittent and jerky and does not regularly follow the long axis of the cell.

Sliding

Bisset (7) in 1939 described strains of streptococci giving rise to colonies almost indistinguishable microscopically from typical medusa-head colonies of *B. anthracis*. Discussing his findings, Bisset states that "the qualities of a colony are merely the aggregate of the qualities of its constituent organisms," and later on, "the growth and consequent extension of the bacterial threads and chains across the surface of the medium is impeded by friction . . ." (7). In his textbook Bisset (8) states: "The long-chained type of streptococcus (also) produces a variety of medusa-head colony," and he comments on the interaction between a tendency to chain formation and the resistance of the surface of the medium. He uses the term "frictionless surface" and describes what would happen on such a surface, were it existing.

The reason for quoting Bisset at length is that his descriptions suggest a phenomenon that comes close to the description I shall give of sliding on the basis of my own observations.

The definition of sliding will also cover colony formation of *B. anthracis* as described as early as in 1910 by Graham-Smith (28) and the formation of colonies of *Kurthia zopfii* with a medusa-head appearance (25).

Spreading zones produced by sliding by different species on different agar plate media (Fig. 19, 21, 23, 25) are macroscopically indistinguishable from spreading zones of twitching strains (Fig. 15 and 17). The corresponding photomicrographs (Fig. 20, 22, 24, and 26 as compared with Fig. 16 and 18) immediately show one important difference: the spreading zone of a sliding organism consists of a single layer of densely packed cells. In the microscope this sheet of cells may be seen to *slide* peripherally without perceptible changes in the mutual disposition of the cells. In other words, movement of single cells, or groups of cells, relative to other cells cannot be observed, and this is the other important difference. Velocity values for the spreading of sliding strains vary from 2 to 5 μm/min up to as much as 25 μm/min with given the strains and the conditions. This should be compared with the colony growth rate values of nonspreading bacteria, e.g., the rate of increase in colony radius (K_r) is 0.33 μm/min for *E. coli* on a defined medium at 37 C (59).

In watching sliding in the microscope, a certain amount of flickering is often noticed in

the field of vision. This effect is produced when individual cells are squeezed, so to say, out of the cell sheet on top of which they thereafter come to rest. The process lasts a fraction of a second and is therefore only perceived as a flickering. Such cells are seen as darker spots in the photomicrographs. Their number evidently decreases peripherally where the generating forces also must be considerably smaller.

Alcaligenes odorans is described as producing spreading surface colonies (27, 51), but the mechanism of this spreading has not been studied in detail before. One reason for this clearly might be that flagella-dependent surface translocation, either swarming or swimming, probably would be an obvious and satisfactory guess to most bacteriologists for this species is peritrichously flagellated. Figures 19 and 20 show a strain of *A. odorans* which is representative of the six strains I have studied of this species. The observations and the pictures are consistent with the description of sliding given above.

In search of gliding motility among strains of *Flavobacterium* sp., spreading was found in 6 out of 30 strains examined, but in all six cases actually was found to be due to sliding (Fig. 21 and 22) (J. Henrichsen, *unpublished observations*). None of 12 strains of *F. meningosepticum* showed any kind of surface translocation (J. Henrichsen, *unpublished observations*). In their study of gram-negative, yellow-pigmented rods, McMeekin et al. (50) in a group of 28 unidentified strains, fitting the general description of *Flavobacterium*, found five strains showing spreading growth but neither twitching nor gliding motility, and they suggest that "this type of swarming could be passive."

Although *A. calcoaceticus* strains, like the one shown in Fig. 23 and 24, have been followed for days in the microscope right from the time of plating, no signs of twitching motility whatsoever were seen and the picture, indeed, was clearly that of sliding.

Until now 12 strains of nonhemolytic streptococci that produce extensive spreading zones have been isolated. They cannot be classified serologically, but biochemically they are all very much alike. One strain is seen in Fig. 25 and 26. Although in appearance quite different from Bisset's medusa-head colonies and independent of chain formation, the sliding of these streptococci may be looked upon as an extreme development of the processes described by Bisset (8). Strangely enough these strains spread nicely on blood agar plates on primary isolation, but in subculture spreading were only seen on AA and never on blood agar.

Extensive spreading due to sliding has also been encountered in a strain of *Moraxella phenylpyruvica* and in a *Corynebacterium* sp.

Fig. 27 shows the quite broad spreading zone of a strain of *B. anthracis* on medium no. 62 after 3 days of incubation at room temperature, and Fig. 28 gives the microscopical picture of the most peripheral part of growth. The production of the long, wavy strands of parallel chains of bacteria plays an important role in the formation of the spreading zone, as was so accurately described by Graham-Smith in 1910 (28), but it fits nicely into the definition of sliding to be given below. Spreading of *B. mycoides* and of *K. zopfii* is essentially similar.

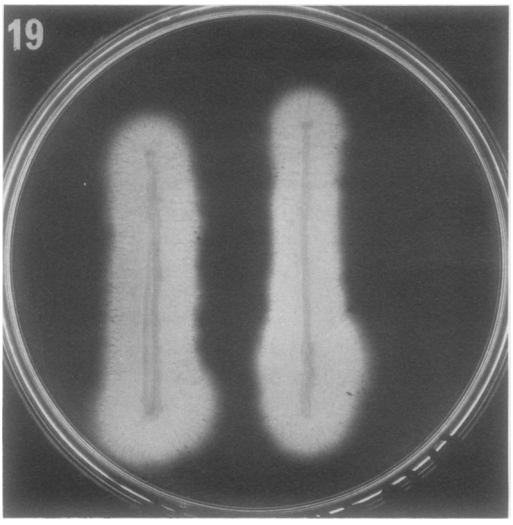
Sliding is not a phenomenon restricted to certain media. It has been met with on all the usual bacteriological media including blood agar plates, but all sliding strains will not slide on any medium. Sliding, as the other kinds of surface translocation, is more or less influenced by the available amount of moisture.

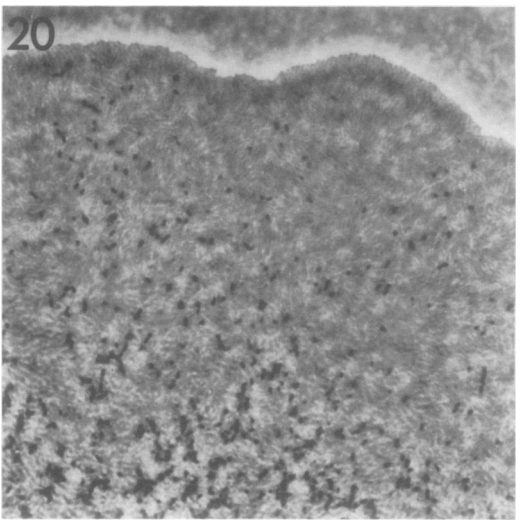
Sliding may be regarded as a special case of the normal process of colony formation of a particular strain on the surface of a particular solid medium. In fact one should expect all bacterial colonies to consist of a circular sheet of cells in one single layer were it not for the forces of friction between the surface of the cell and the surface of the substrate. From this it can be concluded that sliding bacteria must have other surface properties than bacteria that do not slide.

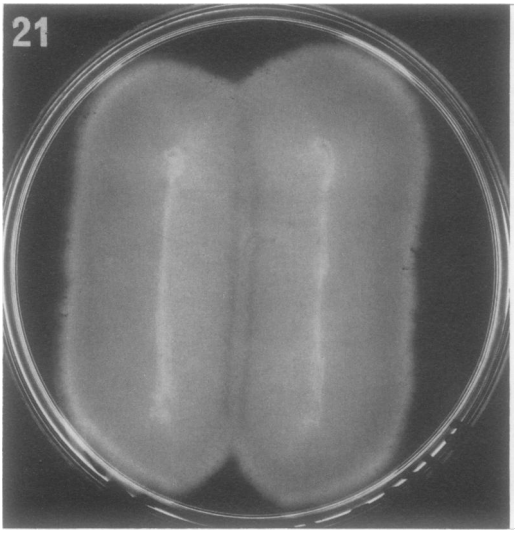
Definition of sliding. Sliding is a kind of surface translocation produced by the expansive forces in a growing culture in combination with special surface properties of the cells resulting in reduced friction between cell and substrate. The micromorphological pattern is that of a uniform sheet of closely packed cells in a single layer. The sheet moves slowly as a unit.

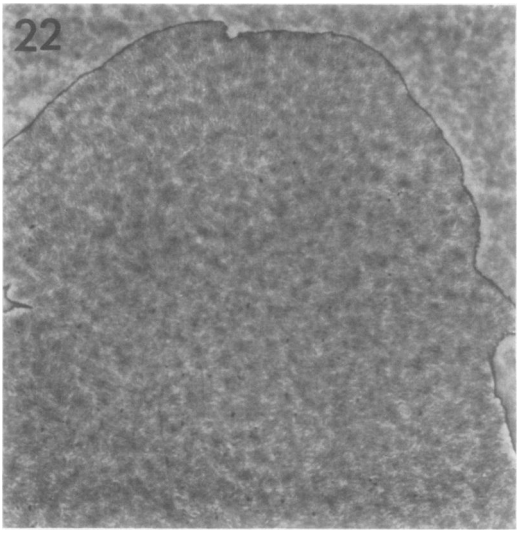
Darting

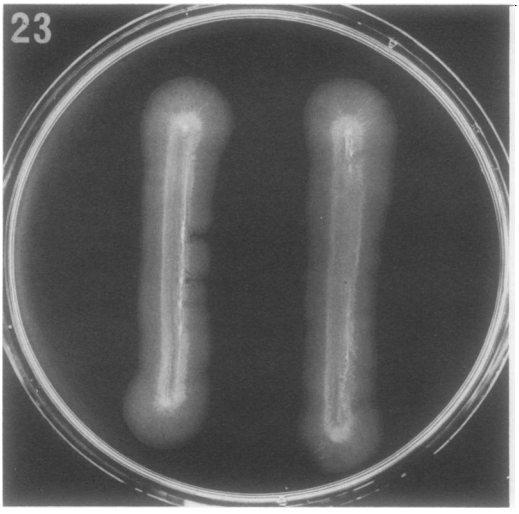
The isolation of spreading staphylococci was reported in 1953 by a Danish veterinary surgeon working on bacteriological control of dairy products and using solid media rather poor in nutrients (43). The spreading colonies were described as looking like a "bacillus colony" and designated "curly variants." On 1,976 "ordinary bacterial count plates," 166 spreading colonies were observed, 109 of which were *Staphylococcus aureus* and 57 *Staphylococcus*

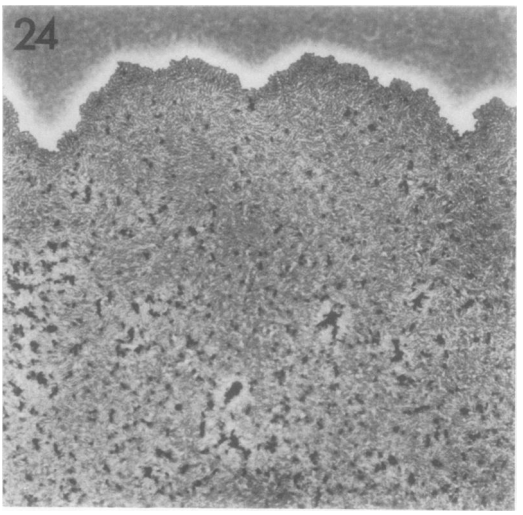












FIGS. 19–24.

albus. After a varying, although rather small, number of subcultures, spreading did not occur any longer. An explanation is not given of the described phenomenon, but it is thought to be related to a particularly vigorous growth.

A spreading *S. albus*, isolated as a contaminant on cytophaga agar no. 70, is shown in Fig. 29. On MEA or blood agar plates spreading is inconspicuous, but the colonies are usually angular rather than circular. Conditions favorable for this type of spreading also include a high atmospheric humidity and, consequently, incubation in plastic bags was used.

Fig. 30 shows that the microscopical pattern of the spreading zone is one of clusters of cocci scattered over the agar surface with cell-free areas in between. Under the microscope motion of some kind is evident, but at first is difficult to analyze. It looks as if "a constant flickering effect (was produced) in the microscopic field," as Puttlitz and Seeley (63) say in their description of probably similar movements observed in strains of *Lampropedia hyalina*. Observing the field carefully this "flickering effect" is eventually seen to be due to pairs of cocci being ejected from the clusters, whereas, in contrast, pairs of cocci lying separately on the agar surface are never seen to move. This would thus seem to indicate that these movements do not depend on contact between cell surface and agar surface and that the explanation should be sought in the conditions within the cell clusters. Hence, the same kind of ejection of cell pairs from clusters ought to be found in ordinary wet mounts. And in point of fact they are.

An explanation of the observed movements of staphylococci could be the following: the cocci are cemented in clusters by a common capsular material or by electrostatic interaction, or both. Counterforces tending to disrupt a given cluster are created as a result of cell multiplication and when these forces become bigger than the cementing forces, a pair of cocci may be "shot" out of the cluster. As for the cell motion of *L. hyalina*, this explanation seems to be consistent with the description given (63).

Definition of darting. Darting is a kind of surface translocation produced by the expansive forces developed in an aggregate of cells inside a common capsule and resulting in the ejection of cells from the aggregate. The micromorphological pattern is that of cells and aggregates of cells distributed at random with empty areas of agar in between. Neither cell pairs nor aggregates move except during the ejection which is observed as a flickering in the microscope.

Classification of the Bacterial Spreading Phenomena

The six different spreading phenomena are easily distinguished by agar plate microscopy, and this direct observation is essential for the classification described in this paper. Swarming and gliding are the only two phenomena that might be difficult to differentiate without further information, for their spreading zones may look similar since motility mainly takes place in rafts. But because swarming is flagella dependent and gliding organisms never are flagellated, a wet mount, perhaps supplemented by a flagella stain, is all that is needed in additional examinations. Twitching and sliding bacteria may or may not be flagellated, but the motive force is in either case independent of flagella. Twitching bacterial cells predominantly move singly and swarming and gliding cells predominantly in aggregates. Sliding bacteria do not move actively, rather they are moved, or pushed, whole sheets of cells together, by centrifugal forces arising from cell multiplication. The motive force of darting is likewise generated in the cell community by growth. A key for the identification of the different spreading phenomena is presented in Table 3. It is based on the following three features that also constitute the main elements of the definitions proposed above: (i) the micromorphological pattern of the spreading zone, including the degree of cell organization;

←FIG. 19. *Alcaligenes odorans strain H 1079 culture plate incubated on meat extract agar at 30 C for 24 hr;* ¾ *natural size.*

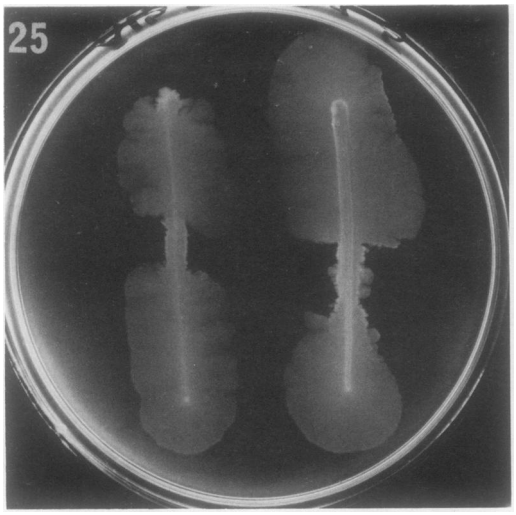
FIG. 20. *Sliding. Peripheral part of spreading zone in Fig. 19 consisting of a densely packed monolayer of cells. The darker spots, increasing in number towards the bottom of the picture, represent cells lying on top of the monolayer; ca.* ×*300.*

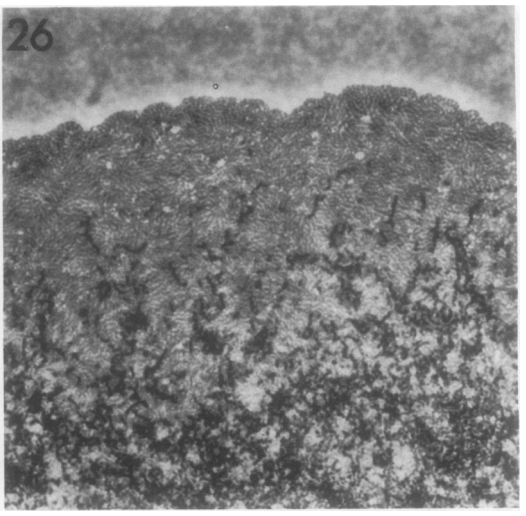
FIG. 21. *Flavobacterium sp. strain U 120 culture plate incubated on meat extract agar at 30 C for 24 hr;* ¾ *natural size.*

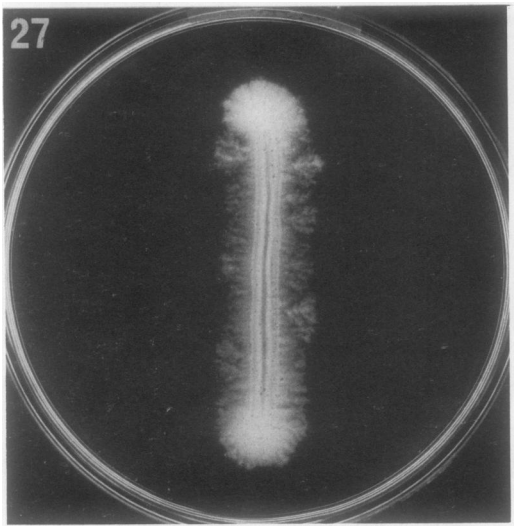
FIG. 22. *Sliding. Peripheral part of spreading zone in Fig. 21 consisting of a very broad monolayer of closely packed cells; ca.* ×*200.*

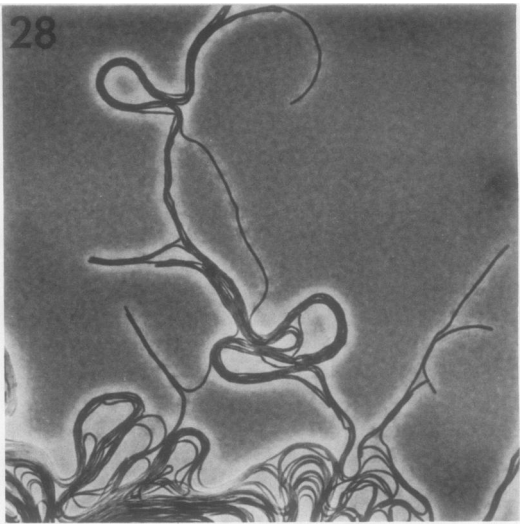
FIG. 23. *Acinetobacter calcoaceticus strain BD-4 culture plate incubated on cytophaga agar no. 62 at 30 C for 24 hr;* ¾ *natural size.*

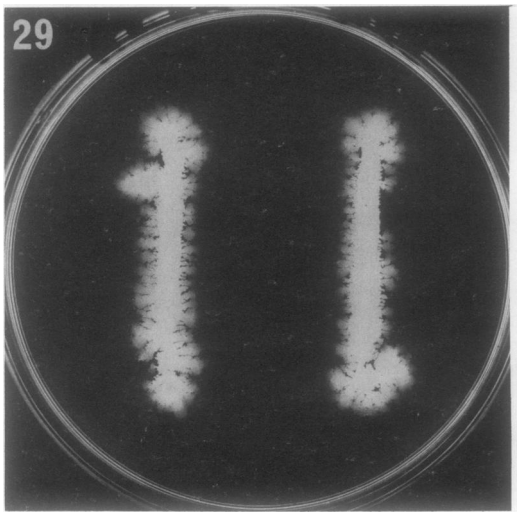
FIG. 24. *Sliding. Peripheral part of spreading zone in Fig. 23; ca.* ×*200.*

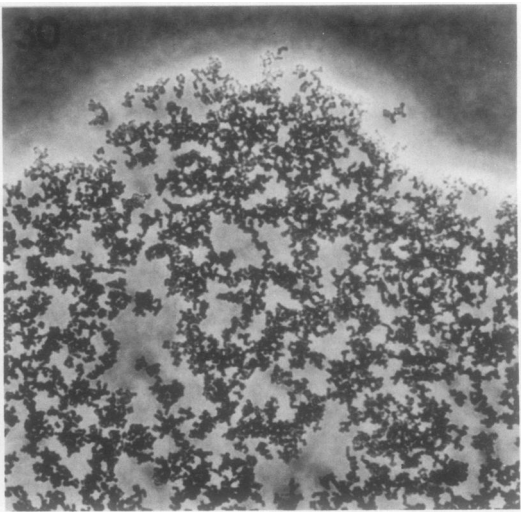












FIGS. 25–30.

(ii) the manner of cell movement; and (iii) the mechanism of movement, whether known or unknown.

CONCLUDING COMMENTS

The value of bacterial spreading as a taxonomic criterion at first sight appears to be difficult to assess, and the existing literature on this subject mirrors the present state of bewilderment (see, e.g., 23, 34, 52, 85). One of the main obstacles obviously is that too little is known about the underlying mechanisms.

TABLE 3. *A key for the identification of kinds of bacterial surface translocation manifested as spreading zones*

Kind of surface translocation	Motive force	
	Flagella	Not flagella
Swarming	Spreading also produced on relatively dry agar surfaces. The cells move together.	
Swimming	Spreading only produced on very moist agar surfaces. The cells move separately.	
Gliding		Motive force generated by the individual cell. High activity. The cells move together.
Twitching		Motive force generated by the individual cell. Low activity. The cells move separately.
Sliding		Motive force generated by the cell community. Centrifugal force in expanding colony.
Darting		Motive force generated by the cell community. Tension force in cell aggregates.

The results presented in this study clearly demonstrate that spreading (swarming in the broad sense) on the surface of solid substrates is not just one phenomenon but may actually be caused by six different kinds of bacterial surface translocation that can be identified with great certainty either as swarming, swimming, gliding and twitching motility, or as sliding and darting motion. Therefore, the value of spreading phenomena in taxonomy should now be reconsidered, especially because gliding motility, often inferred simply from the occurrence of spreading, always has been used as a character of decisive importance in taxonomy. Another obvious consequence of this new knowledge will be an attempt to straighten out the existing inaccuracies in nomenclature.

If the different kinds of spreading phenomena are to be used as taxonomic criteria, the first condition is that each of them be recognized for what it is. For instance, some of the confusion found in the literature concerning the relationship between cytophagas and flavobacteria and the use of gliding motility as a criterion in their distinction may stem from misinterpretations of spreading as being always indicative of gliding. Thus Hayes (34) only observed distinct gliding motility microscopically in 2 out of 20 spreading strains assumed to be cytophagas because of the spreading, and it seems an obvious possibility, in the light of the present investigation and also of that of McMeekin et al. (50), that at least some of these strains actually do not glide but spread by sliding.

Keeping strictly to the definitions given, it can be seen that it is, indeed, different organisms that perform different kinds of surface translocation.

The demonstration of the occurrence of a specific kind of surface translocation in a bacterial strain may then clearly represent a piece of information of some taxonomic value while the opposite kind, i.e., no demonstrable surface translocation, is of much less value because too little is known about the factors controlling the different kinds of spreading.

The taxonomic value of the demonstration of

←FIG. 25. *Streptococcus sp. strain 4932/71 culture plate incubated on ascites agar at 33 C for 72 hr;* ¾ *natural size.*

FIG. 26. *Sliding. Peripheral part of spreading zone in Fig. 25; ca.* ×*300.*

FIG. 27. *Bacillus anthracis strain Ax 11 culture plate incubated on cytophaga agar no. 62 at 22 C for 72 hr;* ¾ *natural size.*

FIG. 28. *Sliding. Peripheral part of spreading zone in Fig. 27; ca.* ×*200.*

FIG. 29. *Staphylococcus albus strain HV 54 culture plate incubated on cytophaga agar no. 70 at 30 C for 24 hr;* ¾ *natural size.*

FIG. 30. *Darting. Peripheral part of spreading zone in Fig. 29; ca.* ×*300.*

swarming, gliding, or twitching depends on the knowledge about the occurrence of these phenomena in different bacterial species. What little is known at present has been given under the appropriate sections above. Briefly summarized, swarming is found in spore-bearing gram-positive species and in two species of *Proteus*; gliding is found in two, also otherwise specialized, groups of gram-negative rods and by definition in cytophagas; and twitching is found among species of *Acinetobacter*, *Moraxella*, and *Pseudomonas*.

Sliding, on the other hand, has been demonstrated in widely different bacterial species, gram-positive as well as gram-negative, and therefore can be of no taxonomic value at the generic level. Whether it may be of some limited value at species level is not known, but some results (e.g., with the streptococci) might suggest so.

Darting most probably will play no role in taxonomy, but a possible value in differentiation at the subspecific level cannot be excluded on the basis of the presently available information.

"Swarming" is an old and accepted term that is still used about any spreading phenomenon, so that a "swarming zone" is generally used to indicate a "spreading zone," no matter how the zone has arisen. It is obvious from the observations of the present study that this nomenclatorial want of precision is no longer acceptable. I therefore suggest that the use of the term "swarming" should be restricted to the motility phenomenon covered by the definition given above for swarming, and that "swarming zone" only should mean a spreading zone produced by swarming in the narrow sense. Furthermore, "swimming" should be used for the flagella-dependent motility seen in wet mounts on insufficiently dried agar plates and in semisolid "motility agar," even if this has been poured into a petri dish, and the medium should not be called "swarm agar."

"Gliding" is an old term, and "twitching" is a relatively recently proposed term. Confusion may be avoided if they are used in accordance with the definitions given above, i.e., twitching should *not* be called a special kind of gliding motility (45).

Furthermore, "sliding" and "darting" are here suggested as new technical terms to designate the two remaining processes described and defined in the present paper. The terms have been chosen because they are considered to reflect a characteristic trait in these two kinds of surface translocation phenomena, just as the terms "gliding" and "twitching" do.

As already mentioned, the use of the term "motility" may give rise to misunderstandings unless the kind of motility in question is specified. Since "motility" traditionally covers flagella-dependent swimming motility, the minimum requirement must be always to specify when swarming, gliding, or twitching motility is at issue. Because the cell motion in sliding and darting strains is basically different, the term "motion" is used here rather than "motility." For the same reason Puttlitz and Seeley (63) write about cell motion of *L. hyalina*.

The expression of all six kinds of spreading phenomena depends on the growth conditions and therefore also on the composition of the medium used. Humidity enhances spreading in all cases. The available moisture is dependent on: (i) the concentration of agar and nutrients; (ii) drying of the plates before use; and (iii) the humidity of the atmosphere in the incubation chamber. In this connection it is worth mentioning that different agars vary greatly with regard to the strength of the gel. Therefore, information about the concentration of agar in a given medium is of no value unless further particulars (e.g., trade name) are given.

The curious phenomenon of motile microcolonies produced by swarming and gliding organisms and by at least one twitching strain seems to be more pronounced when motility is partly inhibited. The motile microcolonies move in curved paths running predominantly counterclockwise in the flagellated organisms, whereas in the nonflagellated organisms there is no predominant direction. An explanation for this has never been found, but a seemingly plausible one is that in the flagellated organisms the direction is determined by the direction of rotation of the flagella relative to the bacterial cell. This hypothesis assumes that flagella rotate, as has been suggested by Vaituzis and Doetsch (83), and that they always, at least within a given organism, rotate in the same direction on all or most cells in a population. Reversal of direction of movement of a cell would not alter the direction of rotation of the flagella relative to the direction of cell movement, for the flagella are assumed to reorientate so that their extremities are always pointing away from the leading part of the cell.

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